UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

January 3, 2017

MEMORANDUM

Subject: Efficacy Review for CaviWipes Bleach; EPA File No. 46781-RU; DP Barcode: D434677

From: Ibrahim Laniyan, Ph.D.
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Regulatory Management Branch II
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Applicant: Metrex Research
28210 Wick Road
Romulus, MI 48174

Formulation from the Label:

Active Ingredients	% by wt.
Sodium Hypochlorite	0.91 %
Other Ingredients:	99. 09 %
Total	100.00 %

(Available Chlorine 8700ppm)

I. BACKGROUND

The product, CaviWipes Bleach (EPA File No. 46781-RU), is a new product. The applicant requested to register the product as a disinfectant (bactericide, fungicide, tuberculocide, virucide, and sporicide against C. difficile) to be applied as towelettes on hard, non-porous surfaces, including food prep, medical, veterinary, dental, and institutional surfaces. Studies were conducted at MicroBioTest Division of Microbac Laboratories, Inc., located at 105 Carpenter Drive Sterling, Virginia 20164, and at Accuratus Lab Services, located at 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

This data package, identified as D434677, contained a letter from the applicant to EPA (dated May 24, 2016), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-27 (Formulator's Exemption), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), thirty-nine studies (MRID Nos. 49921711 through 49921749), Statements of No Data Confidentiality Claims for all studies, and the proposed label (Version 08.2016).

The registrant is seeking Emerging Pathogen Claims on the label as allowed by EPA's 2016 "Guidance to Registrants: Process for Making Claims against Emerging Viral Pathogens Not on EPA Registered Disinfectant Labels".

II. USE DIRECTIONS

The product is designed as a disinfectant to be applied as towelettes for use on hard, non-porous surfaces, including ambulance equipment surfaces, anesthesia equipment, animal equipment, appliances, ATM, baby warmers, baked enamel, bathing units, bidet, biohazard container, BP monitors, cabinets, CAT scan machines, cell phones, center venous lines, chrome, CICU equipment, coated mattresses, coated pillows, counter tops, diaper pails, defibrillators, dental chairs, dental equipment, dental operatory surfaces, desktops, dictating equipment, digital phosphor plates, digital X-Ray sensors, EKG machines, exterior toilet surfaces, external surfaces of ultrasound transducers, eye glasses, food serving trays, Formica, freezers, garbage cans, glass, glazed ceramic tile, glazed porcelain, glazed tiled walls, glazed tiles, glucometers, grocery cart, gurneys, gym equipment, hair cutting implements, hair dryers, hair rollers, hampers, headsets, high chairs, ICU equipment, incubators, infant incubators, infusion pumps, isolettes, IV poles, IV pumps, IV stands, KED, laboratory equipment, life support equipment, light lens covers, lights, linoleum, loupes, Marlite, mattress covers, Mayo, Non-food contact surfaces, operating room furniture, physical therapy surfaces, pipes, plastic laminate, playpens, pneumatic tourniquets, probes, porcelain enamel, radiology equipment, refrigerator carts, respiratory therapy equipment, resuscitators, shelves, shopping carts, shower stalls, sinks, stainless steel, stretches, tables, tanning beds, telephones, toys, and X-ray machines.

Directions on the proposed label provide the following information regarding use of the product:

To [clean and] disinfect [and deodorize] hard, nonporous surfaces:
Wipe hard, nonporous surface to be disinfected. Use enough wipes for treated surface to remain visibly wet for 3 minutes. [Rinse.] Let air dry. Gross filth and heavy soil loads must be removed prior to disinfecting. [If streaking is observed, wipe with a clean, damp [cloth or] paper towel after 3-minute contact time has expired.]

Special Instructions for Cleaning Prior to Disinfection against *Clostridium difficile* spores.

Personal Protection: Wear appropriate barrier protection such as gloves, gowns, masks and eye covering.
Cleaning Procedure: Fecal matter/waste must be thoroughly cleaned from surfaces/objects before disinfection by application with **[Product Name] [this product saturated with product name]**. Cleaning is to include vigorous wiping and/or scrubbing, until all visible soil is removed. Special attention is needed for high-touch surfaces. Surfaces in patient rooms are to be cleaned in an appropriate manner, such as from right to left or left to right, on horizontal surfaces, and top to bottom, on vertical surfaces, to minimize spreading of the spores. Restrooms are to be cleaned last. Do not reuse soiled cloths.
Infectious Materials Disposal: Materials used in the cleaning process that may contain feces/wastes are to be disposed of immediately in accordance with local regulations for infectious materials disposal.

For cleaning and disinfecting the following hard nonporous surfaces: feeding and watering equipment, utensils, instruments, cages, kennels, stables, catteries, etc.
1. Remove all animals and feeds from premises, animal transportation vehicles, crates, cages etc.
2. Remove all litter, droppings and manure from floors, walls and surfaces of facilities (including pens, chutes, and barns) occupied or traversed by animals.
3. Empty all troughs, racks, and other feeding and watering appliances.
4. Thoroughly clean all surfaces with soap or detergent and rinse with potable water. Saturate surfaces by wiping with product for a period of 3 minutes.
5. Wipe all handling and restraining equipment such as leashes, muzzles, halters or ropes, as well as forks, shovels, and scrapers used for removing litter and manure.
6. Ventilate buildings and other closed spaces.
7. Do not house animals or employ equipment until treatment has been absorbed, set or dried.
8. Thoroughly scrub treated feed racks, troughs, mangers, automatic feeders, fountains, and waterers with soap or detergent, and rinse with potable water before reuse.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectant with Sporicidal Activity against *Clostridium difficile*: The Agency has established interim guidance for the efficacy evaluation of antimicrobial products (e.g., dilutable products, ready-to-use products, spray products, vapor, gases, and towelettes) that are labeled for use to treat hard, non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile*. The effectiveness of such a product must be substantiated by data derived from ASTM E 2197: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides. Modifications to each test method will be necessary to specifically accommodate spores of *Clostridium difficile*. Because *Clostridium difficile* is an obligate anaerobe, testing should ensure adequate incubation conditions for the recovery of viable spores. Three product batches should be tested at the lower certified limit(s) (LCL) listed on the confidential statement of formula (CSF) of the product. The toxigenic strains, ATCC 43598, of *Clostridium difficile* must be used for testing. For towelette and spray formulations, the Agency will accept testing of the liquid expressed directly from towelettes or collected directly from spray containers using one of the quantitative methods and conditions specified above. All products should be tested with a 3-part soil load incorporated into the test inoculum by adding 25 µl of 5% bovine serum albumin, 35 µl of 5% yeast extract and 100 µl of 0.4% mucin to 340 µl of the spore suspension. Results must show a minimum 6 log reduction of viable spores in 10 minutes or less. For towelette products, wetness determination test will be used to generate the contact time. Control carrier counts must be greater

than 10^6 spores/carrier. The titer and purity of the final spore preparation must be $>10^8$ spores/mL, and >95% spores. ASTM Standard E2839 specifies procedures for achieving the 95% purity. The acid resistance of purified spores should be assessed against 2.5 M hydrochloric acid (see ASTM Standard E2839). The spores are considered acid-resistant if a log reduction of 0-2 is exhibited following 10 minutes of exposure to 2.5 M HCl.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products Test (for spray products), or the AOAC Hard Surface Carrier Test. The tests require that sixty carriers must be tested with each of 3 samples, representing 3 different product lots at the LCL, against *Staphylococcus aureus* ATCC 6538 (for effectiveness against Gram-positive bacteria), and *Pseudomonas aeruginosa* ATCC 15442 (representative of a nosocomial pathogen), [120 carriers per sample; a total of 360 carriers]. To support products labeled as "disinfectants", killing on 59 out of 60 carriers is required in AOAC Germicidal Spray Products Test to provide effectiveness at the 95% confidence level. To pass performance requirements when using AOAC Hard Surface Carrier Test, tests must result in killing in 58 out of each set of 60 carriers for *Staphylococcus aureus* ATCC 6538; 57 out of each set of 60 carriers for *Pseudomonas aeruginosa* ATCC 15442. Performance requirements when using AOAC Use-Dilution Method are killing in 57 out of each set of 60 carriers for *Staphylococcus aureus* ATCC 6538 and 54 out of each set of 60 carriers for *Pseudomonas aeruginosa* ATCC 15442. Each microbe should be tested three times. Each test should be conducted against a separate batch of product for a total of three batches. Each of the three tests should be conducted on a different day.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the Modified AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using the AOAC Germicidal Spray Products as Disinfectants Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The Modified AOAC Germicidal Spray Products as Disinfectants Method contains procedures for testing fungicidal activity. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity of Disinfectants Test Method): Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain

chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. When using the existing or modified AOAC Tuberculocidal Activity Test Methods, 10 carriers for each of 2 samples, representing 2 different product lots at LCL, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). Killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of Modified AOAC Germicidal Spray Products as Disinfectants Method must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant, at the LCL, must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Virucides - Use of a Surrogate Virus: For certain viruses, there are no *in vitro* systems or *in vivo* animal models (except for humans and chimpanzees). The Agency permits the testing of surrogate viruses in these cases, for example, Bovine Viral Diarrhea virus as a surrogate for human Hepatitis C virus, Duck Hepatitis B virus as a surrogate for Human Hepatitis B virus, and Murine Norovirus/Feline Calicivirus as a surrogate for Norwalk virus.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. **MRID 49921711 "Quantitative Carrier Test to Determine Sporicidal Efficacy Using *Clostridium difficile* Spores", Test Organism: *Clostridium difficile* – spore form (ATCC 43598), for CaviWipes Bleach, EPA File Symbol 46781-RU, by Shirshendu Saha. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – January 5, 2016. Laboratory Project Number: 198-745.**

This study was conducted against *Clostridium difficile* – spore form (ATCC 43598). Three batches (Batch Nos. 15-1323RDO-W, 15-1348RDO-W, and 15-2348RDO-W) of the product, CaviWipes Bleach, were tested using protocol Identification Number 198-745 (copy provided). The test substance was ready to use (RTU) impregnated towelette, as received from the Sponsor. Just prior to testing, the liquid was extracted (aseptically) into a tube for the use in the testing. Three CDC Anaerobic Blood Agar (CABA) plates were streaked with thawed vegetative frozen stock culture. Two plates were incubated anaerobically and the third one was incubated aerobically at 36±1°C for 48±4 hours. If no growth was observed in the plate incubated aerobically,

a pure colony from the CABA plates incubated anaerobically was used to inoculate 10 mL of pre-reduced RCM broth. The broth culture was mixed well by vortexting and incubated anaerobically at 36±1°C for 24±2 hours. Ten CABA plates were incubated using the Reinforced clostridial medium (RCM) broth culture (100µL/plate) and incubated anaerobically for 10 days at 36±1°C and approximately 70% relative humidity. All the plates were sealed to prevent dehydration. After 24±2 hours of incubation, one plate was inspected for confluent growth. On day 10 of incubation, the plates were placed inside a Biological Safety Cabinet (BSC). The culture was examined for conversion to sporulation. The culture was considered ready for harvesting once ≥90% spore conversion has occurred. The culture was harvested from each plate by adding 5.0 mL of Phosphate Buffered Saline with 0.1% Tween 80 (PBS-T) to each plate and gently scraping the surface of each plate to dislodge the spore. The harvested material was divided into two 50 mL conical tubes, which were centrifuged at 4500xg for 15 minutes. The pellicle was resuspended in 30 mL of PBS-T. The pellet was then disaggregated by vortex mixing, which was considered the first was. After the third wash, the supernatant was discarded and the pellet was resuspended in 4 mL of PBS-T. The spore suspension was heat treated in a heat block for 10±1 minutes at 65±2°C, the cooled at room temperature. The spore suspension was microscopically evaluated for predominantly spores using we-mount techniques under a phrase-contrast microscope. The titer of the spore suspension went through serial 10-fold dilutions to 10-7 in PBS-T and selected dilution was spread plated (0.1 mL) on Recovery medium, Brain Heart Infusion Yeast Extract with Horse Blood and Sodium Taurocholate (BHIY-HT) in duplicate. All plates were inverted and incubated anaerobically at 36±1°C for 24±4 hours. Additional spore purification was performed using 50% (w/v) solution of HistoDens in sterile deionized water and incubated. The purified spore suspension was tested for HCL resistance using 990 µL of 2.5 M HCL and incubated. The prepared purified spore suspension was stored in small aliquots (e.g. 500 µL) in cyrovials at -20±1C for up to 1 year for use in testing). The culture contained organic soil load using the following ratios per 500 µL final test suspension: 340 µL of the prepared spore suspension, 25 µL Bovine Serum Albumin, 100 µL Bovine Mucin, and 35 µL of Yeast Extract.

Up to 20 sterile stainless steel disks were transferred to a sterile Petri dish. Ten (10) µL of culture was placed in the center of each disk using a calibrated positive displacement pipettor. After all disks in the Petri dish were inoculated, the carriers were dried in a biosafety hood for 30±5 minutes with the Petri dish. The contaminated carriers were placed in a desiccator connected to a vacuum line. A vacuum was drawn and the carriers continued to dry under vacuum for 2 hours under room temperature. The dried carriers were used within 24 hours of inoculation. Each contaminated and dried carrier was transferred inoculated side up to the surface of a Teflon vial. Each lot was evaluated with the test substance on independent test days. In a timely fashion, ten carriers containing the dried inoculum were covered with fifty µL of the test substance. The treated carriers were equilibrated to 20-25°C. The test carriers were held at 20-25°C for the contact period of 3 minutes and in 37-39% relative humidity. Following the exposure time, 10.0 ml of neutralizer (Phosphate Buffered Saline (PBS) + 0.5% Sodium Thiosulfate) was added to the vial containing the carrier, which was then vortex mixed for approximately 2-3 seconds. Dilution was initiated within 30 minutes at room temperature after neutralization. Filtration was performed using 0.2µm polyethersulfone (PES) filters. The vial contents (10^0 dilution) were briefly vortex mixed and 1mL was removed to prepare serial dilutions in 9 mL PBS-T out to a minimum of the 10^{-1} dilution. Each tube was rinsed once with ~10 mL of PBS, briefly vortex mixed and poured into the same filter unit. The inside surface of each filter unit was rinsed with additional ~20 PBS with the vacuum on. Additional washing and filtering were repeated. The membrane filter was aseptically removed and placed on the recovery medium (pre-reduced BHIY-HT). Each sealed package was opened inside the BSC just prior to placement of the membrane filter. All the control plates were incubated for 48±4 hours and all the test plates were incubated for 72±4 hours with an additional 48±4 hours if no or few growth was observed after initial incubation. Study controls

included neutralization confirmation, spore titer, carrier population, and culture purity control. The reported controls for each lot in CFU/carrier for ***Clostridium difficile* – spore form are 6.32 log_{10}, 6.35 log_{10}, and 6.36 log_{10}.**

2. **MRID 49921712 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Healthcare", Test Organism: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella enterica* (ATCC 10708) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Kelsey Roach. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – February 16, 2016. Laboratory Project Number: 198-769.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella enterica* (ATCC 10708). Three batches (Batch Nos. 15-1348RDO-W, 15-2348RDO-W, and 16-1008RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-769 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of synthetic broth (*Staphylococcus aureus* and *Salmonella enterica*) or nutrient broth (*Pseudomonas aeruginosa*), mixed and incubated for 24±2 hours at 35-37°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The *Pseudomonas* culture was not vortex mixed. The pellicle formed in the *Pseudomonas* culture was removed prior to carrier contamination. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Sixty (60) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 30 minutes at 35°C and 52 - 59% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20-21°C) for 3 minutes at 35% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 35-37°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

3. **MRID 49921713 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Burkholderia cepacia*", Test Organism: *Burkholderia cepacia* (ATCC 25416) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis**

Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 04, 2016. Laboratory Project Number: 198-777.

This study was conducted against *Burkholderia cepacia* (ATCC 25416). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-777 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Trypticase Soy Broth, mixed and incubated for 24±2 hours at 25-30°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 35 minutes at 37°C and 40 – 41% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 29-30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 25-30°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible" should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

4. **MRID 49921714 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Escherichia coli* O157:H7", Test Organism: *Escherichia coli* O157:H7 (ATCC 35150) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 04, 2016. Laboratory Project Number: 198-778.**

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-778 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred

to an initial 10 mL tube of Trypticase Soy Broth, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 35 minutes at 37°C and 40 – 41% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 29-30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:
Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible" should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

5. **MRID 49921715 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Enterobacter cloacae*", Test Organism: *Enterobacter cloacae* (NDM-1 positive) [CDC strain 1000654], ATCC BAA-2468 for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 04, 2016 (Study amended date – May, 12, 2016). Laboratory Project Number: 198-770.**

This study was conducted against *Enterobacter cloacae* (NDM-1 positive) [CDC strain 1000654], ATCC BAA-2468. Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-770 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Trypticase Soy Broth, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The

test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 35 minutes at 37°C and 40 – 41% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 29-30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

6. **MRID 49921716 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Klebsiella pneumoniae*", Test Organism: *Klebsiella pneumoniae* (ATCC 4352) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 04, 2016. Laboratory Project Number: 198-779.**

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-779 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Trypticase Soy Broth, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were

dried for 35 minutes at 37°C and 40 – 41% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 29-30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

7. **MRID 49921717 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Legionella pneumophila*", Test Organism: *Legionella pneumophila* (ATCC 33153) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 25, 2016 (Study amended date – May 12, 2016). Laboratory Project Number: 198-780.**

This study was conducted against *Legionella pneumophila* (ATCC 33153). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-780 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Charcoal Yeast Extract Broth, mixed and incubated for 24±2 hours at 36±1°C and ~5% CO_2. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C and ~5% CO_2. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 38 minutes at 36-37°C and 25% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was

pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Charcoal Yeast Extract Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C and ~5% CO_2. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

8. **MRID 49921718 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Listeria monocytogenes*", Test Organism: *Listeria monocytogenes* (ATCC 19117) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 25, 2016. Laboratory Project Number: 198-781.**

This study was conducted against *Listeria monocytogenes* (ATCC 19117). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-781 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Brain Heart Infusion Broth, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 38 minutes at 36-37°C and 25% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back

and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

9. **MRID 49921719 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Serratia marcescens*", Test Organism: *Serratia marcescens* (ATCC 14756) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 3, 2016 (Study amended date – May 12, 2016). Laboratory Project Number: 198-782.**

This study was conducted against *Serratia marcescens* (ATCC 14756). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-782 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Tryptic Soy Broth, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 40 minutes at 36°C and 35% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for

wiping the last five carriers. Each treated carrier was held at room temperature (21°C) for 3 minutes at 26-27% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

10. **MRID 49921720 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Streptococcus pyogenes*", Test Organism: *Streptococcus pyogenes* (ATCC 12344) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 25, 2016. Laboratory Project Number: 198-783.**

This study was conducted against *Streptococcus pyogenes* (ATCC 12344). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-782 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Tryptic Soy Broth containing 5% Defibrinated Sheep's Blood, mixed and incubated for 24±2 hours at 36±1°C and ~5% CO_2. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C and ~5% CO_2. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 38 minutes at 36-37°C and 25% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate and 5% Defibrinated Sheep's Blood. All subcultures were incubated for 48±2 hours at 36±1°C and ~5% CO_2. All observations

were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

11. **MRID 49921721 "Pre-Saturated Towelettes for Hard Surface Disinfection", Test Organism: *Neisseria gonorrhoeae* (ATCC 43069) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Jamie Herzan. Study conducted at Accuratus Lab Services, Study completion date – March 28, 2016. Project Number A20425.**

This study was conducted against *Neisseria gonorrhoeae* (ATCC 43069). Two batches (Batch Nos. 16-1041RDO-W and 16-1042RDO-W) of the product, CaviWipes Bleach, were tested using Accuratus Lab Services Protocol No. MET02012816.TOW (copy provided). The product was received as a ready to use (RTU) towelettes. The towelette was folded in half lengthwise twice and rolled up five times prior to use. A culture of test organism was prepared by using a stock culture to inoculate multiple agar plates and incubating for 3 days at 35-37°C in CO_2. The growth medium used was Chocolate agar. Following incubation, an organism suspension was prepared in Fluid Thioglycollate Medium to target 1 x 10^8 CFU/mL. A spectrophotometer reading of 1.241 at 620 nm was prepared to target this range. A 0.10 mL aliquot of FBS was added to 1.90 mL of broth culture to yield a 5% fetal bovine serum organic soil load. Ten (10) glass slide carriers per product batch were inoculated with 10.0 µL of test organism suspension using a pipettor. Inoculum was spread over approximately a 1-inch x 1-inch area of the slide contained in the Petri dish. The slides were dried for 30 minutes at 25-30°C and 65% relative humidity, and were used within 2 hours of drying. One towelette was used to wipe the contaminated portions of 10 carriers. The area of the towelette used was rotated so as to expose a maximum amount of the towelette surface during the course of the wiping procedure. Each inoculated carrier was treated with the towelette by passing over the carrier surface back and forth three times for a total of 6 passes. The treated carriers were held for 3-minute exposure time at room temperature (20°C) and 46% relative humidity in a horizontal and undisturbed fashion. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to a volume of neutralizing subculture medium (Letheen Broth with 0.1% Sodium Thiosulfate) sufficient to completely cover the inoculated and treated area (20mL). The subcultures were vortex mixed, and within 30 minutes of mixing, the entire volume of the subculture broths were individually transferred to the surface of a filter membrane pre-wetted with 10 mL of sterile saline and filtered using a vacuum pump. Each filter membrane was washed with ≥50 mL of sterile saline. Each filter membrane was removed aseptaicly from the filter unit and placed on the surface of a Chocolate Agar plate for recovery of the test organism. All subcultures were incubated for 2-4 days at 35-37°C in CO_2. Following incubation and storage, the subcultures were examined for growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population control.

12. **MRID 49921722 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental Multi-Drug Resistant *Acinetobacter baumannii*", Test Organism: Drug-Resistant *Acinetobacter baumannii* (ATCC BAA-1605) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date –**

March 3, 2016 (Study amended date – May 12, 2016). Laboratory Project Number: 198-771.

This study was conducted against Multi-Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-771 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Trypticase Soy Broth containing 10 mcg/mL Ceftazidime, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 40 minutes at 36°C and 35% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (21°C) for 3 minutes at 26-27% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, verification of antibiotic resistance, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

Project Sheet No. 2, Study Director Signature line was inadvertently not signed.

13. **MRID 49921723 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental Carbapenem-Resistant *Klebsiella pneumoniae*", Test Organism: Carbapenem-Resistant *Klebsiella pneumoniae* (ATCC BAA-1705) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 4, 2016. Laboratory Project Number: 198-772.**

This study was conducted against Carbapenem-Resistant *Klebsiella pneumoniae* (ATCC BAA-1705). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-772 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Tryptic Soy Broth, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 35 minutes at 37°C and 40-41% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 29-30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, verification of antibiotic resistance, bacteriostasis, and confirmation of challenge microorganisms.

Note:

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14. **MRID 49921724 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental Methicillin-Resistant *Staphylococcus aureus*", Test Organism: Methicillin-Resistant *Staphylococcus aureus* (ATCC 33592) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 4, 2016. Laboratory Project Number: 198-773.**

This study was conducted against Methicillin-Resistant *Staphylococcus aureus* (ATCC 33592). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-773 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Tryptic Soy Broth, mixed and incubated

for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 35 minutes at 37°C and 40-41% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 29-30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, verification of antibiotic resistance, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

15. **MRID 49921725 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Streptococcus pneumoniae*", Test Organism: *Streptococcus pneumoniae* (ATCC 700677) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 25, 2016. Laboratory Project Number: 198-774.**

This study was conducted against *Streptococcus pneumoniae* (ATCC 700677). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-774 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Trypticase Soy Broth containing 5% Defibrinated Sheep's Blood, mixed and incubated for 24±2 hours at 36±1°C and ~5% CO_2. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C and ~5% CO_2. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then

removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 38 minutes at 36-37°C and 25% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate and 5% Defibrinated Sheep's Blood. All subcultures were incubated for 48±2 hours at 36±1°C and ~5% CO_2. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, verification of antibiotic resistance (penicillin), bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

16. **MRID 49921726 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental Vancomycin-Resistant *Staphylococcus aureus*", Test Organism: Vancomycin-Resistant *Staphylococcus aureus* (NARSA VRS1) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 25, 2016. Laboratory Project Number: 198-775.**

This study was conducted against Vancomycin-Resistant *Staphylococcus aureus* (NARSA VRS1). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-775 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Brain Heart Infusion Broth containing 4 mcg/mL Vancomycin, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism.

Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 38 minutes at 36-37°C and 25% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (20°C) for 3 minutes at 30% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, verification of antibiotic resistance, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

17. **MRID 49921727 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental Vancomycin-Resistant *Enterococcus faecalis*", Test Organism: Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51575) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 3, 2016. Laboratory Project Number: 198-776.**

This study was conducted against Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51575). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-776 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Brain Heart Infusion Broth containing 4 mcg/mL Vancomycin, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 40 minutes at 36°C and 35% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10

replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (21°C) for 3 minutes at 26-27% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, verification of antibiotic resistance, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

18. **MRID 49921728 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental Extended spectrum β-lactamase *Escherichia coli*", Test Organism: Extended spectrum β-lactamase *Escherichia coli* (ATCC BAA-196) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 3, 2016 (Amended completion date – May 12, 2016). Laboratory Project Number: 198-806.**

This study was conducted against Extended spectrum β-lactamase *Escherichia coli* (ATCC BAA-196). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-806 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Tryptic Soy Broth with 10 mcg/mL Ceftazidime, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 40 minutes at 36°C and 35% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each

canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (21°C) for 3 minutes at 26-27% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, verification of antibiotic resistance, bacteriostasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

Project Sheet No. 2, Study Director Signature line was inadvertently not signed.

19. **MRID 49921729 "Testing Pre-Saturated or Impregnated Towelettes for Tuberculocidal Effectiveness", Test Organism: *Mycobacterium bovis* - BCG; OrganonTeknika, Corp. for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 27, 2016 (Amended completion date – April 29, 2016). Laboratory Project Number: 198-746.**

Two batches (Batch Nos. 15-1321RDO-W and 15-1323RDO-W) of the product, CaviWipes Bleach, were tested against *Mycobacterium bovis* - BCG; OrganonTeknika, Corp. using Lab Protocol No. 198-746. From a stock culture of test organism, tubes containing 20 mL of Modified Proskauer-Beck Medium (MPB) were inoculated with 1 or 2 loopfuls of organism from 7H11 slants at 36±1°C while remaining quiescent for 22±2 days. Sufficient growth was transferred from the surface of the culture into a sterile tissue grinder. One mL of 0.85% NaCl with 0.1% Polysorbate 80 was added and the culture was macerated to break up large clumps. Nine mL of MPB was added to the homogenized culture. This suspension was transferred to a sterile tube and the culture was allowed to settle for 10-15 minutes. The upper portion of the culture was removed and transferred to a sterile flask, leaving behind any debris or clumps. Using a spectrophotometer, the culture suspension was standardized using MPB to achieve 20% transmittance (T) at 650 nm. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 30 minutes at 36°C and 34-37% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded

lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (21°C) for 3 minutes at 25% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Heat Inactivated Donor Horse Serum containing 0.5% Sodium Thiosulfate. Each tube containing carrier in neutralizer was thoroughly shaken and the carrier was transferred to a tube containing 20 mL of MPB broth within 5-10 minutes. From each tube of neutralizer, two mL was subcultured to a tube containing 20 mL of 7H9 broth and 2 mL was subcultured to a tube containing 20 mL Kirchner's medium. All test tubes used for secondary transfers (MPB, 7H9, and KM) and all control tubes were incubated incubated at 36±1°C. Observations for growth or no growth was made on days 21, 45, 60, and 90 of incubation[1]. All plates were incubated for 17-21 days at 36±1°C, the colonies were counted and the average CFU calculated. On the day of the final reading, acid-fast stains were performed for all test culture tubes demonstrating visible growth and two viability control tubes in order to verify the presence of the challenge microorganism[2]. Culture morphology was also observed. In addition, isolation streaks from the viabilities and one randomly selected positive tube was performed on 7H9 or 7H11 agar and incubate for 17-21 days at 36±1°C. Following incubation, the plates were evaluated for colony morphology and acid fast stained. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, performance assessment of Media, and confirmation of challenge microorganisms.

Note:

Protocol Amendment:

Due to availability, the secondary test lot (Lot No. 15-1322RDO-W) in the Miscellaneous Information section of the protocol will be replaced with Lot No. 15-1323RDO-W as outlined above.

The cover page of the protocol states, "Confidential MicroBioTest Protocol" this is a typographical error and should only state "MicroBioTest Protocol".

[2]Page 9 of the protocol indicates that acid-fast stains will be performed for all test culture tubes demonstrating visible growth and two viability control tubes. For clarification, page 9 should state that all viability media tubes demonstrating visible growth will be streaked for isolation prior to being acid-fast stained to verify the presence of the challenge microorganism.

[1]Protocol Deviations: Page 9 of the protocol indicates observation for growth or no growth will be made on days 21, 45, 60 and 90 of incubation. However, observation was only recorded for days 60 and 90. This departure had no effect on the study.

20. **MRID 49921730 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Candida albicans*", Test Organism: *Candida albicans* (ATCC 10231) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Travis Farley. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 3, 2016 (Amended completion date – May 12, 2016). Laboratory Project Number: 198-784.**

This study was conducted against *Candida albicans* (ATCC 10231). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-784 (copy provided). A 10 µL aliquot of a thawed stock organism was transferred to an initial 10 mL tube of Tryptic Soy Broth, mixed and incubated for 24±2 hours at 36±1°C. Daily transfers were made for at least one but no more than five consecutive days. The final test culture was incubated for 48-54 hours at 35-37°C. The test cultures were agitated on a Vortex type mixer for 3 to 4 seconds and allowed to stand for 10 minutes. The upper portion of each culture was then removed, leaving behind any clumps or debris and was pooled in a sterile flask. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 40 minutes at 36°C and 35% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (21°C) for 3 minutes at 26-27% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Letheen Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 48±2 hours at 36±1°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, fungistasis, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

Project Sheet No. 2, Study Director Signature line was inadvertently not signed.

21. **MRID 49921731 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Aspergillus brasiliensis*", Test Organism: *Aspergillus brasiliensis* (ATCC 16404) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Kelsey Roach. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 16, 2016 (Amended completion date – May 11, 2016). Laboratory Project Number: 198-785.**

This study was conducted against *Aspergillus brasiliensis* (ATCC 16404). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-785 (copy provided). The fungus was inoculated from stock culture onto Potato

Dextrose Agar and incubated for ≥10 but ≤15 days at 25-30°C or until sporulation. The mycelial mats were removed from the surface of at least five plates and macerated with sterile saline + 0.05% Triton X (SS+) in a tissue grinder. The suspension was filtered through sterile glass wool to remove the hyphae. The density of the conidial suspensions was determined by standard plate count techniques. The plates were incubated for 3-5 days at 25-30°C. The suspension was stored at 2-10°C for ≤4 weeks before use. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 30 minutes at 36°C and 30-31% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (19-20°C) for 3 minutes at 25-26% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Potato Dextrose Broth with 0.2% Sodium Thiosulfate. All subcultures were incubated for 10 days at 25-30°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, inoculum counts control, fungistasis control, and confirmation of challenge microorganisms.

Note:

Protocol Amendment: Page 6 of the protocol has a typographical error, for clarification Section D (Test) "The slide should touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible should be "The slide may touch both the interior sides of the Petri dish and the neutralizer tube during the transfer, but care will be taken to avoid this contact as much as possible".

22. **MRID 49921732 "Testing Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection Supplemental *Trichophyton mentagrophytes*", Test Organism: *Trichophyton mentagrophytes* (ATCC 9533) for CaviWipes Bleach, EPA File Symbol 46781-RU,** by **Kelsey Roach. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 27, 2016 (Amended completion date – April 29, 2016). Laboratory Project Number: 198-786.**

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two batches (Batch Nos. 16-1027RDO-W and 16-1029RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-786 (copy provided). The fungus was inoculated from stock culture onto Neopeptone Glucose Agar[3] and incubated for ≥10 but ≤15 days at 25-30°C or until sporulation. The mycelial mats were removed from the surface of at least five plates and macerated with sterile saline + 0.05% Triton X (SS+)[4] in a tissue grinder. The suspension was filtered through sterile glass wool to remove the hyphae. The density of the conidial suspensions was determined by

standard plate count techniques. The plates were incubated for 3-5 days at 25-30°C. The suspension was stored at 2-10°C for ≤4 weeks before use. Heat activated fetal bovine serum was added to each culture to achieve an organic load of 5%. Ten (10) glass microscope slide carriers per product batch were inoculated with 0.01 mL of test microorganism. Inoculum was spread over a 1 square inch area of the slide. The carriers were dried for 30 minutes at 36°C and 38-40% relative humidity, and were used within 2 hours of drying. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. For each group of 10 replicates, a single towelette was used based on the following: The lid of each canister was removed and the center of the roll pulled out and inserted into the lid opening. The lid was replaced and the towelette was pulled through the lid opening. The first five wipes were removed from each canister and discarded before testing. Initially, the towelette was folded lengthwise twice then five times inward. Then the outside edges were pulled inward to form a U shape and grasped on one side with the thumb and on the other side with the index and middle finger. The folded towelette was rotated 90°C. Each contaminated carrier was wiped back and forth three times lengthwise with the towelette for a total of six passes across the inoculum. The used end was flipped upward towards self, reoriented and then used to wipe the next four carriers, folding the used portion up-and-over each time. Once five carriers were wiped, the second lengthwise fold was unfolded and refolded in the opposite direction. The towelette was refolded five times as before and the above procedure for wiping the first five carriers was repeated for wiping the last five carriers. Each treated carrier was held at room temperature (19°C) for 3 minutes at 60-61% relative humidity. Following the exposure time, the excess liquid was drained from the carriers and the carriers were transferred to 20 mL Neopeptone Glucose Broth containing 0.2% Sodium Thiosulfate. All subcultures were incubated for 10 days at 25-30°C. All observations were recorded as growth or no growth. Controls included those of sterility, viability, neutralization effectiveness, carrier counts, inoculum counts control, fungistasis control, and confirmation of challenge microorganisms.

Note:

[3]Because one of the reagents needed to prepare the Neopeptone Glucose Agar is not available from the supplier at this time, Sabouraud Dextrose Agar will be used for the control plates.

[4]The protocol states that sterile saline+ 0.05% Triton X (SS+) should be used to harvest the *Trichophyton mentagrophytes.* However, 0.85% saline solution (SS) will be used instead.

23. **MRID 49921733 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Adenovirus Type 2", Test Organism: Adenovirus Type 2, Strain Adenoid 6, ATCC VR-846 for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 8, 2016. Laboratory Project Number: 198-799.**

This study was conducted against Adenovirus Type 2, Strain Adenoid 6, ATCC VR-846. Cultures of A549 cells, ATCC CCL-185, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-799 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (21°C) for 30 minutes at 29.8 – 30.8% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times.

The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new once was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (21°C) for 3 minutes at 30.7 – 30.8% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in DMEM + 2% FBS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 11-14 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

24. **MRID 49921734 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Hepatitis A Virus", Test Organism: Hepatitis A Virus, University of Ottawa for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-801.**

This study was conducted against Hepatitis A Virus, Strain HM175/18f, University of Ottawa. Cultures of FRhK-4 cells, University of Ottawa, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-801 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 25.5 – 25.8% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new once was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated

carrier was held at room temperature (20°C) for 3 minutes at 25.0 – 25.8% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in DMEM + 2% FBS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 16-21 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

25. **MRID 49921735 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Feline calicivirus (Surrogate for Human Norovirus)", Test Organism: Feline calicivirus (ATCC VR-782) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-802.**

This study was conducted against Feline calicivirus, Strain F9, ATCC VR-782. Cultures of CrFK cells (ATCC CCL-94) were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-801 (copy provided). Two glass carriers with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 40.0 – 40.3% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new once was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 39.9 – 40.0% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of RPMI 1640 (RPMI) + 10% Newborn Calf Serum (NCS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in RPMI + 2% NCS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 33±2°C[5] with 5±1% CO_2 for total 7-9 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for

presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

Note:

[5]Protocol Amendment: Page 10 of the protocol states the incubation temperature as 33±2°C. It should be 36±2°C.

26. **MRID 49921736 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Poliovirus", Test Organism: Poliovirus Type 1 Strain Chat (ATCC VR-1562) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – March 2, 2016. Laboratory Project Number: 198-828.**

This study was conducted against Poliovirus Type 1 Strain Chat (ATCC VR-1562). Cultures of MA-104 cells, Charles River Laboratories, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 15-1348RDO-W and 15-2348RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-828 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (21°C) for 30 minutes at 35.2 – 35.9% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new once was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (21°C) for 3 minutes at 35.1 – 35.6% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of RPMI 1640 (RPMI) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in RPMI + 2% FBS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 6-9 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

27. **MRID 49921737 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Rhinovirus Type 37", Test Organism: Rhinovirus Type 37**

(ATCC VR-1147) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-804.

This study was conducted against Rhinovirus Type 37, Strain 151-1 (ATCC VR-1147). Cultures of H1-Hela cells, ATCC CRL-1958, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-804 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 26.0-26.1% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 26.1 – 26.2% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of RPMI 1640 (RPMI) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in RPMI + 2% FBS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 33±2°C with 5±1% CO_2 for total 6-9 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

28. **MRID 49921738 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Enterovirus EV-D68", Test Organism: Enterovirus EV-D68 (ATCC VR-561) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 21, 2016. Laboratory Project Number: 198-805.**

This study was conducted against Enterovirus EV-D68 (ATCC VR-561). Cultures of Vero cells, ATCC CCL-81, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-805 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square

inches. Carriers treated with virus were dried at room temperature (20-21°C) for 30 minutes at 30.1-30.8% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 29.5 – 30.1% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of Minimum Essential Medium (MEM) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in MEM + 0.5µg/mL Trypsin. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 9-12 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

29. **MRID 49921739 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Human Rotavirus", Test Organism: Human Rotavirus, Strain WA, (ATCC VR-2018) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 08, 2016. Laboratory Project Number: 198-807.**

This study was conducted against Human Rotavirus, Strain WA, (ATCC VR-2018). Cultures of MA-104 cells, Charles River Laboratories, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-807 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 42.9 – 43.3% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped

using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 42.9 – 43.0% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of Minimum Essential Medium (MEM) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in MEM + 1.0µg/mL Trypsin. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 33±2°C[6] with 5±1% CO_2 for total 5-7 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

Note:

[6]Protocol Amendment: Protocol page 10 states that the incubation temperature is 33±2°C. The correct incubation temperature is 36±2°C. This amendment serves to correct the typographical error on protocol page

30. **MRID 49921740 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Herpes Simplex Virus Type 1", Test Organism: Herpes Simplex Virus Type 1, (ATCC VR-260) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-791.**

This study was conducted against Herpes Simplex Virus Type 1 (ATCC VR-260). Cultures of Vero cells, ATCC CCL-81, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-791 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 39.7 – 46.5% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 39.7 – 40.5% relative humidity. Upon completion of the

contact time, the virus and test substance mixture was neutralized with 1.0 mL of RPMI 1640 (RPMI) + 10% Newborn Calf Serum (NCS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in RPMI 1640 + 2% NCS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 5-8 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

31. **MRID 49921741 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Herpes Simplex Virus Type 2", Test Organism: Herpes Simplex Virus Type 2, Strain G (ATCC VR-734) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 08, 2016. Laboratory Project Number: 198-792.**

This study was conducted against Herpes Simplex Virus Type 2, Strain G (ATCC VR-734). Cultures of Vero cells, ATCC CCL-81, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-792 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 35.5– 42.0% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 34.9 – 35.5% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of RPMI 1640 (RPMI) + 10% Newborn Calf Serum (NCS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in RPMI 1640 + 2% NCS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 5-8 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL

($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

32. **MRID 49921742 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Human Coronavirus", Test Organism: Human Coronavirus, Strain 229E (ATCC VR-740) for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-793.**

This study was conducted against Human Coronavirus, Strain 229E (ATCC VR-740). Cultures of MRC-5 cells, ATCC CCL-171, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-793 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 36.4 – 36.7% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 36.1 – 36.4% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of 1x Minimum Essential Medium (MEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in MEM + 5% FBS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 33±2°C with 5±1% CO_2 for total 5-7 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

33. **MRID 49921743 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Influenza A", Test Organism: Influenza A Virus (H3N2), Strain A/Hong Kong/8/68, Charles River Laboratories for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division**

of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-794.

This study was conducted against Influenza A Virus (H3N2), Strain A/Hong Kong/8/68, Charles River Laboratories. Cultures of MDCK cells, ATCC CCL-34, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-793 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 35.3 – 35.6% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 35.6 – 41.9% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of Minimum Essential Medium (MEM) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in MEM + 3.0 µg/mL Trypsin. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 4-6 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

34. **MRID 49921744 "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection Virus: Influenza B virus" for CaviWipes Bleach, EPA File Symbol 46781-RU, by Melissa Bruner. Study conducted at Accuratus Lab Services, Study completion date – March 30, 2016. Project Number: A20451.**

This study was conducted against Influenza B virus, ATCC VR-823, Strain B/Hong Kong/5/72, obtained from the American Type Culture Collection. Cultures of MDCK (canine kidney) (ATCC CCL-34) were obtained from the American Type Culture Collection and were used as the host system. Two batches (Batch Nos. 16-1041RDO-W and 16-1042RDO-W) of the product, CaviWipes Bleach, were tested according to Accuratus Lab Services Protocol No. MET02012816.FLUB. The virus stock was adjusted to contain 5% fetal bovine serum as the organic soil load. The product was received as single use towelettes impregnated with the active ingredient. The towelettes were folded widthwise 2 times and lengthwise 3 times to form a square

for use in testing. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C and 40% relative humidity. For each batch of test substance, the dried virus film was wiped with one saturated towelette and held covered at room temperature of 20.0°C for the 3-minute exposure time. Each carrier was treated in sections. Each section was treated by wiping the virus film with the towelette over and back three times for a total of six passes. The area of the towelette used for wiping was rotated so as to expose a maximum amount of its surface in the course of wiping each section of the carrier. Following the exposure time, a 2.0 mL aliquot of test medium was added to each petri dish, and the dishes were scraped with a plastic cell scraper to resuspend the contents (10-1 dilution). The mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers. The filtrates (10-1 dilution) were then tittered by 10-fold serial dilution and each dilution was assayed for infectivity and/or cytotoxicity. The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2 µg/mL TPCK-Trypsin, 25 mM HEPES, 0.2% bovine serum albumin (BSA) fraction V, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µ/mL amphotericin B. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 11 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

35 **MRID 49921745 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Duck Hepatitis B Virus (Surrogate for Human Hepatitis B Virus)", Test Organism: Duck Hepatitis B Virus, Strain Grimaud, HepadnaVirus Testing for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 27, 2016. Laboratory Project Number: 198-796.**

This study was conducted against Duck Hepatitis B Virus, Strain Grimaud, HepadnaVirus Testing. Cultures Primary Duck Hepatocyte cells; Metzer Farms (Duckling source) were used as the host system. The virus inoculum contained 100% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-796 (copy provided). Two glass carriers with approximately 10-square inch surface area were prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (21°C) for 30 minutes at 44.1 – 45.3% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (21°C) for 3 minutes at 45.2 –

46.4% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of L-15 Complete + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in L-Complete. The residual infectious virus in both test and controls were detected by immunofluorescent staining targeting the S envelop protein of DHBV (DHBsAg). Dilutions of the neutralized inoculum/test agent mixtures were selected based on the projected virus titration end point and were inoculated onto Primary duck hepatocytes (four wells per dilution per reaction mixture) and incubated at 36±2°C with 5±1% CO_2 for total 20-30 hours for viral adsorption. After adsorption, the monolayer was refed with medium and returned to the same incubation conditions for a total of 10-14 days. After incubation the infectious DHBV were assayed by immunofluorescence assay according to MicroBioTest SOP M1006.VI.013. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

36 MRID 49921746 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus)", Test Organism: Bovine Viral Diarrhea Virus, Strain NADL for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-797.

This study was conducted against Bovine Viral Diarrhea Virus, Strain NADL, American BioResearch Labs. Cultures MDBK cells, ATCC CCL-22, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-797 (copy provided). Two glass carriers with approximately 10-square inch surface area were prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (21°C) for 30 minutes at 45.4 – 53.5% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (21°C) for 3 minutes at 45.7 – 46.2% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of 1X Minimum Essential Medium (MEM) + 10% Horse Serum (HS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used

for making serial 10-fold dilutions in MEM + 2% HS. The residual infectious virus in both test and controls were detected by viral-induced cytopathic effect (CPE). Selected dilutions of the neutralized inoculum/disinfectant test substance mixture were added to cultured host cells (four wells per dilution per reaction mixture) and incubated at 33±2°C with 5±1% CO_2 for total 6-9 days. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

Note:
Protocol Amendment: Page 3 and 4 of the Protocol list the source of the challenge virus as "ATCC VR-1422" and the incubation temperature as "36±2°C". The correct listing should be "American BioResearch Labs" and "33±2°C", respectively. This amendment serves to correct the typographical error in the Protocol.
Protocol Amendment No. 1 on Project sheet No. states, "Page 3 and 4 of the Protocol list the source ... and the incubation temperature as '36±2°C': The correct listing should be ... '33±2°C' ... ". However, the amendment should read "Page 3 of the' Protocol lists the source of the challenge virus as 'ATCC VR-1422' and Page 10 lists the incubation temperature as '33±2°C'. The correct listing should be 'American BioResearch Labs' and '36±2°C ', respectively ... ". This amendment serves to correct the typographical error of Amendment No.1 on Project Sheet No. 1.

37 MRID 49921747 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Human Immunodeficiency Virus Type 1", Test Organism: Human Immunodeficiency Virus Type 1, Strain IIIB (B), ZeptoMetrix for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016. Laboratory Project Number: 198-798.

This study was conducted against Human Immunodeficiency Virus Type 1, Strain IIIB (B), ZeptoMetrix. Cultures of C8166, University of Pennsylvania, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-793 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 31.6– 33.2% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 31.6 – 32.4% relative

humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of RPMI 1640 + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in RPMI 1640 + 2% FBS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 9-12 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

38 **MRID 49921748 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Respiratory Syncytial Virus", Test Organism: Respiratory Syncytial Virus, Strain Long, ATCC VR-26 for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 21, 2016. Laboratory Project Number: 198-826.**

This study was conducted against Respiratory Syncytial Virus, Strain Long, ATCC VR-26. Cultures of HeLa cells, ATCC CCL-2, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-793 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (20°C) for 30 minutes at 30.5 – 41.3% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (20°C) for 3 minutes at 32.5 – 41.3% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Bovine Serum (FBS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in DMEM + 2% FBS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 14-18 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The 50% tissue culture infectious dose

per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

39 MRID 49921749 "Virucidal Hard Surface Efficacy Test for Pre-Saturated or Impregnated Towelettes – Canine Parvovirus", Test Organism: Canine Parvovirus, Cornell 780916-80 Strain, ATCC VR-2017 for CaviWipes Bleach, EPA File Symbol 46781-RU, by Zheng Chen. Study conducted at MicroBioTest Division of Microbac Laboratories, Inc., Study completion date – April 11, 2016 (Amended final report date – May 9, 2016). Laboratory Project Number: 198-800.

This study was conducted against Canine Parvovirus, Cornell 780916-80 Strain, ATCC VR-2017. Cultures of CrFK cells, ATCC CCL-94, were used as the host system. The virus inoculum contained 5% serum. Two batches (Batch Nos. 16-1032RDO-W and 16-1039RDO-W) of the product, CaviWipes Bleach, were tested using protocol No. 198-793 (copy provided). One glass carrier with approximately 10-square inch surface area was prepared for each lot of the test substance. For each carrier, an aliquot of 1.0 mL virus was added and spread with a cell scraper over the entire area of 10 square inches. Carriers treated with virus were dried at room temperature (21°C) for 30 minutes at 26.6 – 27.2% relative humidity. The canisters containing the towelettes were inverted to re-saturate the towelettes just prior to use. The first towelette from the center of the roll through the opening in the lid was pulled out. A minimum of 3 towelettes was discarded prior to using any towelettes for testing. Each piece of towelette was folded lengthwise in half two times. The far end was folded up once and the folding was repeated in the same direction so that an additional 4 folds of the same size were made resulting in a total of 5 folds. The outside edges were pulled inward to form a U shape when wiping. Handling of the towelette was minimal and no liquid was expressed during the folding. If a substantial amount of liquid was expressed during folding, the towelette was discarded and a new one was used. Following the drying of the virus, each carrier was wipes using one piece of towelette. The carriers were wiped using three passes, where 1 pass equaled a back and forth motion for a total of 6 motions. The carrier was treated in sections to ensure sufficient contact of the entire contact area. Since the carrier was treated in sections, a new unused area of the folded towelette was exposed for each section. The sections were treated in such a way that overlapping was minimal. Each treated carrier was held at room temperature (21°C) for 3 minutes at 27.3 – 27.6% relative humidity. Upon completion of the contact time, the virus and test substance mixture was neutralized with 1.0 mL of RPMI 1640 + 10% Newborn Calf Serum (NCS) + 0.5% Polysorbate 80 + 3% HEPES + 0.025N HCl + 0.5% $Na_2S_2O_3$. The mixture was then scraped off from the surface with a cell scraper. The post-neutralized sample (PNS) was undiluted. An aliquot of the PNS was used for making serial 10-fold dilutions in RPMI 1640 + 2% NCS. Selected dilutions of the sample were added to cultured cell monolayers at a minimum of four wells per dilution per sample and incubated at 36±2°C with 5±1% CO_2 for total 5-7 days. The inoculated culture was observed and refed with fresh media as necessary during the incubation period. The host cells were examined microscopically for presence of infectious virus. The presence of infectious virus was determined by immunofluorescence staining (FA). The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman-Karber. Controls included those for plate recovery, neutralizer effectiveness, cytotoxicity control, cell viability, virus stock titer, and column titer control (if applicable).

V. RESULTS

MRID 499217-11

Organism	Contact Time	Batch No.	Geometric Mean of Test Carriers (Av. Log_{10})	Geometric Mean of Population Control Carriers (Av. Log_{10})	Percent Reduction (Log_{10} Reduction)
Clostridium difficile – spore form (ATCC 43598)	3 minutes	15-1323RDO-W	0.22	6.32	6.10
		15-1348RDO-W	0.08	6.35	6.27
		15-2348RDO-W	0.20	6.36	6.16

Spore Purity of all three lots were 99% pure, which indicates passing results.

MRID Number	Organism	Media	No. Exhibiting Growth/ Total No. Tested	
			Lot 15-1321RDO-W 90 Days	Lot 15-1323RDO-W 90 Days
3 minutes Exposure Time				
499217-29	*Mycobacterium bovis* BCG Carrier Population: 1.0 X 10^5 CFU/carrier	Modified Proskauer-Beck Medium	0/10	0/10
		Middlebrook 7H9 Broth	0/10	0/10
		Kirchner's Medium	0/10	0/10

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Counts (CFU/ carrier)
		Lot 15-1348RDO-W	Lot 15-2348RDO-W	Lot 16-1008RDO-W	
3 minutes Exposure Time					
499217-12	*Staphylococcus aureus*	1/60	0/60	1/60	3.23 x 10^6
	Salmonella enterica	0/60	0/60	1/60	3.33 x 10^4
	Pseudomonas aeruginosa	1/60	1/60	1/60	2.267 x 10^6
		Lot 16-1027RDO-W	**Lot 16-1029RDO-W**		
499217-13	*Burkholderia cepacia*	0/10	0/10	---	1.4 X 10^5
499217-14	*Escherichia coli* O157:H7	0/10	0/10	---	3.7 X 10^6
499217-15	*Enterobacter cloacae* (NDM-1 positive) [CDC strain 1000654]	0/10	0/10	---	6.4 X 10^4
499217-16	*Klebsiella pneumoniae*	0/10	0/10	---	9.6 X 10^5
499217-17	*Legionella pneumophila*	0/10	0/10	---	2.0 X 10^4
499217-18	*Listeria monocytogenes*	0/10	0/10	---	1.3 X 10^4
499217-19	*Serratia marcescens*	0/10	0/10	---	2.6 X 10^5
499217-20	*Streptococcus pyogenes*	0/10	0/10	---	2.2 X 10^4
499217-22	Multi-Drug Resistant *Acinetobacter baumannii*	0/10	0/10	---	1.5 X 10^5

499217-23	Carbapenem-Resistant *Klebsiella pneumoniae*	0/10	0/10	---	1.6×10^5
499217-24	Methicillin-Resistant *Staphylococcus aureus*	0/10	0/10	---	2.7×10^5
499217-25	*Streptococcus pneumoniae*	0/10	0/10	---	2.3×10^4
499217-26	Vancomycin-Resistant *Staphylococcus aureus*	0/10	0/10	---	9.7×10^5
499217-27	Vancomycin-Resistant *Enterococcus faecalis*	0/10	0/10	---	1.2×10^6
499217-28	Extended spectrum β-lactamase *Escherichia coli*	0/10	0/10	---	2.8×10^4
499217-30	*Candida albicans*	0/10	0/10	---	2.2×10^4
499217-31	*Aspergillus brasiliensis*	0/10	0/10	---	1.1×10^4
499217-32	*Trichophyton mentagrophytes*	0/10	0/10	---	1.4×10^4
		Lot 16-1041RDO-W	**Lot 16-1042RDO-W**		
499217-21	*Neisseria gonorrhoeae*	0/10	0/10	---	8.3×10^4

MRID Number	**Organism**	**Description**	**Results**		**Dried Virus Control** ($TCID_{50}$/Carrier)
			Lot 16-1032RDO-W	**Lot 16-1039RDO-W**	
3 minutes Exposure Time					
499217-33	Adenovirus Type 2	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.30}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log Reduction	≥4.50	≥4.50	
499217-34	Hepatitis A Virus	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.50}$
		$TCID_{50}$/carrier	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log Reduction	≥6.00	≥6.00	
499217-35	Feline Calicivirus (Surrogate for Human Norovirus)	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{5.57}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log Reduction	≥3.77	≥3.77	
499217-37	Rhinovirus Type 37	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.80}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log Reduction	≥5.00	≥5.00	
499217-38	Enterovirus EV-D68	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{6.75}$
		10^{-2} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	

		$TCID_{50}$/carrier	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		Log Reduction	≥5.25	≥5.25	
499217-39	Human Rotavirus	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{4.75}$
		$TCID_{50}$/carrier	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log Reduction	≥4.25	≥4.25	
499217-40	Herpes Simplex Virus Type 1	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{7.93}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log Reduction	≥6.13	≥6.13	
499217-41	Herpes Simplex Virus Type 2	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.18}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log Reduction	≥4.38	≥4.38	
499217-42	Human Coronavirus	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.25}$
		$TCID_{50}$/carrier	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log Reduction	≥5.75	≥5.75	
499217-43	Influenza A Virus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.00}$
		10^{-2} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	
		$TCID_{50}$/carrier	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		Log Reduction	≥3.50	≥3.50	
499217-45	Duck Hepatitis B Virus (Human Hepatitis B Virus)	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{5.39}$
		$TCID_{50}$/carrier	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		Log Reduction	≥3.89	≥3.89	
499217-46	Bovine Viral Diarrhea Virus (Human Hepatitis C Virus)	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.37}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log Reduction	≥4.57	≥4.57	
499217-47	Human Immunodeficiency Virus Type 1	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{7.55}$
		10^{-2} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	
		$TCID_{50}$/carrier	$\leq 10^{2.80}$	$\leq 10^{2.80}$	
		Log Reduction	≥4.75	≥4.75	
499217-48	Respiratory Syncytial Virus	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.43}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	

		Log Reduction	≥4.63	≥4.63	
499217-49	Canine Parvovirus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.00}$
		10^{-2} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	
		$TCID_{50}$/carrier	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		Log Reduction	≥3.50	≥3.50	
			Lot 15-1348RDO-W	**Lot 15-2348RDO-W**	
499217-36	Poliovirus Type 1, Strain Chat	10^{-1} - 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	$10^{6.05}$
		$TCID_{50}$/carrier	$\leq 10^{1.80}$	$\leq 10^{1.80}$	
		Log Reduction	≥4.25	≥4.25	
			Lot 16-1041RDO-W	**Lot 16-1042RDO-W**	**Dried Virus Control ($TCID_{50}$/100 µl)**
499217-44	Influenza B virus	10^{-1} - 10^{-7} dilutions	Complete Inactivation	Complete Inactivation	$10^{5.50}$
		$TCID_{50}$/100 µl	$\leq 10^{0.50}$	$\leq 10^{0.50}$	
		Log Reduction	≥5.00	≥5.00	

VI. CONCLUSIONS

1. The submitted efficacy data **support** the use of the product, CaviWipes Bleach (EPA File No. 46781-RU), as a disinfectant with sporicidal activity against *Clostridium difficile* – spore (ATCC 43598) on pre-cleaned, hard, non-porous surfaces for 3-minute contact time at room temperature.

2. The submitted efficacy data **support** the use of the product, CaviWipes Bleach (EPA File No. 46781-RU), as a disinfectant with bactericidal activity against the following microorganisms on visibly clean, hard, non-porous surfaces for 3 minute contact time at room temperature:

Staphylococcus aureus (ATCC 6538) MRID 499217-12
Salmonella enterica (ATCC 10708) MRID 499217-12
Pseudomonas aeruginosa (ATCC 15442) MRID 499217-12
Burkholderia cepacia (ATCC 25416) MRID 499217-13
Escherichia coli O157:H7 (ATCC 35150) MRID 499217-14
Enterobacter cloacae (NDM-1 positive) [CDC strain 1000654], ATCC BAA-2468 MRID 499217-15
Klebsiella pneumoniae (ATCC 4352) MRID 499217-16
Legionella pneumophila (ATCC 33153) MRID 499217-17
Listeria monocytogenes (ATCC 19117) MRID 499217-18
Serratia marcescens (ATCC 14756) MRID 499217-19
Streptococcus pyogenes (ATCC 12344) MRID 499217-20
Neisseria gonorrhoeae (ATCC 43069) MRID 499217-21
Multi-Drug Resistant *Acinetobacter baumannii* (ATCC BAA-1605) MRID 499217-22
Carbapenem-Resistant *Klebsiella pneumoniae* (ATCC BAA-1705) MRID 499217-23

Methicillin-Resistant *Staphylococcus aureus* (ATCC 33592)	MRID 499217-24
Streptococcus pneumoniae (ATCC 700677)	MRID 499217-25
Vancomycin-Resistant *Staphylococcus aureus* (NARSA VRS1)	MRID 499217-26
Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51575)	MRID 499217-27
Extended spectrum β-lactamase *Escherichia coli* (ATCC BAA-196)	MRID 499217-28

3. The submitted efficacy data (MRID 499217-29) **support** the use of the product, CaviWipes Bleach (EPA File No. 46781-RU), as a disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on visibly clean, hard, non-porous surfaces for 3 minute contact time.

4. The submitted efficacy data **support** the use of the product, CaviWipes Bleach (EPA File No. 46781-RU), as a disinfectant with fungicidal activity against the following microorganisms on visibly clean, hard, non-porous surfaces for a 3 minute contact time.

Candida albicans (ATCC 10231)	MRID 499217-30
Aspergillus brasiliensis (ATCC 16404)	MRID 499217-31
Trichophyton mentagrophytes (ATCC 9533)	MRID 499217-32

5. The submitted efficacy data **support** the use of the product, CaviWipes Bleach, as a disinfectant with virucidal activity against the following microorganisms on visibly clean, hard, non-porous surfaces for 3 minute contact time:

Adenovirus Type 2, Strain Adenoid 6 (ATCC VR-846)	MRID 499217-33
Hepatitis A Virus, Strain HM175/18f	MRID 499217-34
Feline Calicivirus (FCV), Strain: F9 (ATCC VR-782)	MRID 499217-35
Poliovirus Type 1, Strain Chat (ATCC VR-1562)	MRID 499217-36
Rhinovirus Type 37, Strain 151-1 (ATCC VR-1147)	MRID 499217-37
Enterovirus EV-D68 (ATCC VR-561)	MRID 499217-38
Human Rotavirus, Strain WA, (ATCC VR-2018)	MRID 499217-39
Herpes Simplex Virus Type 1 (ATCC VR-260)	MRID 499217-40
Herpes Simplex Virus Type 2, Strain G (ATCC VR-734)	MRID 499217-41
Human Coronavirus, Strain 229E (ATCC VR-740)	MRID 499217-42
Influenza A Virus (H3N2), Strain A/Hong Kong/8/68	MRID 499217-43
Influenza B virus, Strain B/Hong Kong/5/72 (ATCC VR-823)	MRID 499217-44
Duck Hepatitis B Virus, Strain Grimaud	MRID 499217-45
Bovine Viral Diarrhea Virus, Strain NADL	MRID 499217-46
Human Immunodeficiency Virus Type 1, Strain IIIB (B)	MRID 499217-47
Respiratory Syncytial Virus, Strain: Long (ATCC VR-26)	MRID 499217-48
Canine Parvovirus, Cornell 780916-80 Strain (ATCC VR-2017)	MRID 499217-49

VII. LABEL

1. The proposed label claims that the product, CaviWipes Bleach (EPA File No. 46781-RU), is an effective disinfectant with sporicidal activity against *Clostridium difficile* – spore (ATCC 43598) on pre-cleaned, hard, non-porous surfaces for 3-minute contact time at room temperature.

2. The proposed label claims that the product, CaviWipes Bleach (EPA File No. 46781-RU), is an effective disinfectant against the following microorganisms on visibly clean, hard, non-porous surfaces for a 3-minute contact time at room temperature:

Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708)
Pseudomonas aeruginosa (ATCC 15442)
Burkholderia cepacia (ATCC 25416)
Escherichia coli O157:H7 (ATCC 35150)
Enterobacter cloacae (NDM-1 positive) [CDC strain 1000654], ATCC BAA-2468
Klebsiella pneumoniae (ATCC 4352)
Legionella pneumophila (ATCC 33153)
Listeria monocytogenes (ATCC 19117)
Serratia marcescens (ATCC 14756)
Streptococcus pyogenes (ATCC 12344)
Neisseria gonorrhoeae (ATCC 43069)
Multi-Drug Resistant (MDR) *Acinetobacter baumannii* (ATCC BAA-1605) (**Resistant to Ceftazidime and Gentamicin**)
Carbapenem-Resistant *Klebsiella pneumoniae* (ATCC BAA-1705)
Methicillin-Resistant *Staphylococcus aureus* (MRSA) (ATCC 33592)
Streptococcus pneumoniae (ATCC 700677)
Vancomycin-Resistant *Staphylococcus aureus* (NARSA VRS1)
Vancomycin-Resistant *Enterococcus faecalis* (ATCC 51575)
Extended spectrum β-lactamase (ESBL) *Escherichia coli* (ATCC BAA-196) (**Resistant to Ceftazidime and Penicillin**).
Mycobacterium bovis, var: bovis (BCG)
Candida albicans (ATCC 10231)
Aspergillus brasiliensis (ATCC 16404)
Trichophyton interdigitale (ATCC 9533)
Adenovirus Type 2, Strain Adenoid 6 (ATCC VR-846)
Hepatitis A Virus, Strain HM175/18f
Norovirus (Norwalk Virus) [Feline Calicivirus (FCV), Strain: F9 (ATCC VR-782)]
Poliovirus Type 1, Chat Strain (ATCC VR-1562)
Rhinovirus Type 37, Strain 151-1 (ATCC VR-1147)
Enterovirus EV-D68 (ATCC VR-561)
Human Rotavirus, Strain WA, (ATCC VR-2018)
Herpes Simplex Virus Type 1 (ATCC VR-260)
Herpes Simplex Virus Type 2, Strain G (ATCC VR-734)
Human Coronavirus, Strain 229E (ATCC VR-740)
Influenza A Virus (H3N2), Strain A/Hong Kong/8/68
Influenza B virus, Strain B/Hong Kong/5/72 (ATCC VR-823)
Duck Hepatitis B Virus, Strain Grimaud (Surrogate for Human Hepatitis B Virus)
Bovine Viral Diarrhea Virus, Strain NADL (Surrogate for Human Hepatitis C Virus)
Human Immunodeficiency Virus Type 1, Strain IIIB (B)
Respiratory Syncytial Virus, Strain: Long (ATCC VR-26)
Canine Parvovirus, Cornell 780916-80 Strain (ATCC VR-2017)

These claims are acceptable as they are supported by the submitted data. Registrant must qualify "Multi-Drug" and "Extended spectrum β-lactamase" list the drugs associated with them: Ceftazidime and Gentamicin for Multi-Drug Resistant *Acinetobacter baumannii* and, Ceftazidime and Penicillin for Extended spectrum β-lactamase *Escherichia coli.*

3. The following revision must be made to the proposed label:

- Remove all "decontaminant", "decontaminating", "decontaminate", "decontamination"

claims on the label.

- On page 3 of the label, revise the first paragraph in the emerging pathogens text box to read as follows: "This product qualifies for emerging pathogens claims against **enveloped, large non-enveloped, and small non-enveloped** viruses when used according to the directions for use against feline Calicivirus."

- On page 7, 14, and 15 of the label, replace "Multidrug/multi drug Resistant" with "Drug-Resistant" in "Multidrug-Resistant Bacteria". The list is not made of only multidrug-resistant organisms.

- Correct the name of the organism "*Trichophyton* ***mentagrophytes***" to reflect "*Trichophyton* ***interdigitale*** (ATCC 9533)".

- On page 7 of the label, spell out "ESBL" as "Extended spectrum β-lactamase" before initials (see in (VII. 2.) part of this review).

On page 9 and 11 of the label, remove bacteria ([*Burkholderia cepacia*], [*Escherichia coli* O157:H7, *Pseudomonas aeruginosa*], [Hepatitis A [Virus] [Human[[(HAV)], [Herpes simplex virus [type 1], and [*Staphylococcus aureus*]) from the list of 6 [six] log] reduction organisms.

- Remove all "Advanced", "Perfect", "Prevent the spread", "Ideal", "a unique" claims and "[To fight Clostridium difficile¥ -or- C. difficile¥ -or- C. diff¥ [(C. **diff**)]¥ [spores] outbreaks]]" because they are heightened terms and statement.

- Remove all "More manageable", "Easier", "Simpler", "Bigger", "Better", "Thinker", "Stronger", "Larger" "X% more", "X% reduction", "X% less material", "by X%" claims or specifically name your own product that this product is being compared to.

- On pages 1, 10 and 13 of the label, revise all claims regarding 1:10 dilutions as follows:

  - remove the term "requirements"
  - remove the CDC website links
  - remove references to sodium hypochlorite concentrations not equivalent to the product concentration (such as 1000 ppm, 5250-6150 ppm)

- On page 9 of the label, remove "Sporicidal" claim.

- On page 10 and 11 of the label, remove "Does not contain toxic chemicals" claims.

- On page 10 and 19 of the label, remove "the risk" from "reduces **the risk** of cross-contamination" claims and add "**on treated**" before listing surfaces.

- Qualify all "one-step", "one step", "one easy step" claims to indicate the exception for *C. difficile*, HIV-1, HBV and HCV.

- Add asterisk to all general "viruses" claims as it should.

- On page 12 of the label, remove "Dries quickly" claim or give the exact drying time; this is not even helping in the effectiveness of the towelettes.

- On page 13 of the label, remove "99.99% of"; **only "99.9% of" is acceptable in this case.**

- On page 14 of the label, remove the statements "Intermediate level disinfectant" and "Just wipe to clean and disinfect."

- On page 15 of the label, remove "**Leave nothing behind**" claim.

- On page 17 and 19 of the label, remove "reduces exposure" and "reduces transmission" claims.

- On page 18 of the label, add "treated" prior to "surfaces" in "[This product] controls the spread of germs on most hard, nonporous surfaces".

- On page 19 of the label, add "...on **treated** surfaces" in "[To reduce Clostridium difficile¥ -or- C. difficile¥ -or- C. diff¥][(C. diff)]¥ [spores] **on surfaces** in [▲]" and "[To kill Clostridium difficile¥ -or- C. difficile¥ -or- C. diff¥ [(C. diff)]¥ [spores][,] especially during outbreaks [of Clostridium difficile¥ -or- C. difficile¥ -or- C. diff¥ [(C. diff)]¥] [let stand for three -or- 3 min[utes]]"

- On page 25 of the label, add "hard non-porous surfaces" to the title of Table 7: "CaviWipes Bleach are recommended for use on [♦]"

- On page 28, use terms "Exterior surfaces of Toilet".